

## Ionized Hypercalcemia in Dogs: A Retrospective Study of 109 Cases (1998–2003)

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**Background:** Serum hypercalcemia in dogs has been reported in association with a variety of diseases. Serum-ionized calcium (iCa) concentration is a more accurate measure of hypercalcemia than total serum calcium or corrected serum calcium concentrations. The severity of hypercalcemia has been utilized to suggest the most likely differential diagnosis for the hypercalcemia.

**Hypothesis:** Diseases causing ionized hypercalcemia may be different than those that cause increases in total or corrected serum calcium concentrations. The severity of ionized hypercalcemia in specific diseases cannot be used to determine the most likely differential diagnosis for ionized hypercalcemia.

**Animals:** One-hundred and nine client-owned dogs with a definitive cause for their ionized hypercalcemia evaluated between 1998 and 2003 were included in this study.

**Methods:** Retrospective, medical records review.

**Results:** Neoplasia, specifically lymphosarcoma, followed by renal failure, hyperparathyroidism, and hypoadrenocorticism were the most common causes of ionized hypercalcemia. Dogs with lymphoma and anal sac adenocarcinoma have higher serum iCa concentrations than those with renal failure, hypoadrenocorticism, and other types of neoplasia. The magnitude of serum-ionized hypercalcemia did not predict specific disease states.

**Conclusions and Clinical Importance:** Serum-ionized hypercalcemia was most commonly associated with neoplasia, specifically lymphosarcoma. Although dogs with lymphosarcoma and anal sac adenocarcinoma had higher serum iCa concentrations than dogs with other diseases, the magnitude of the serum iCa concentration could not be used to predict the cause of hypercalcemia. Total serum calcium and corrected calcium concentrations did not accurately reflect the calcium status of the dogs in this study.

**Key words:** Hypervitaminosis D; Hypoadrenocorticism; Lymphosarcoma; Primary hyperparathyroidism.

Serum total calcium (Ca) exists in 3 fractions: protein-bound, complexed (to phosphate, citrate, sulfate, lactate, or bicarbonate), and ionized.<sup>1</sup> Ionized calcium (iCa), which comprises approximately 50% of serum total Ca, is the most biologically active form and directly affects parathyroid hormone (PTH) and vitamin D release to regulate body Ca concentrations.<sup>2</sup> Ionized Ca is a cofactor in many vital enzymatic reactions and regulates extra- and intracellular signaling pathways. Thus, it is a major controller of many cellular functions. Because of its importance, iCa concentration is tightly regulated in the blood. Major regulators of serum iCa concentrations are vitamin D, PTH, and calcitonin. Abnormal serum iCa concentration can be a sensitive indicator of pathological states.<sup>3–5</sup>

The practitioner may be hampered in the recognition of a potentially pathologic disturbance in Ca status in the patient because most routine serum chemistry profiles report serum total Ca concentrations only. These results

include the protein-bound and complexed forms of Ca as well as the biologically active ionized form. Correlations between ionized and total Ca concentrations can be inaccurate, thereby not reflecting the true Ca status of the animal.<sup>6</sup> To address this problem, equations have been derived in dogs that attempt to correlate total serum Ca to iCa concentrations, but these have proven inaccurate.<sup>7,8</sup> In recent years, the use of portable chemistry analyzers that measure iCa has become more routine in veterinary medicine. This practice has allowed collection of data reflecting accurate Ca status in association with disease states in veterinary practice.

Previous studies have attempted to predict the most common causes of hypercalcemia in the dog, and most have implicated neoplasia in over 50% of cases.<sup>9–12</sup> These retrospective studies were performed examining serum total Ca concentration which may not accurately reflect the true Ca status of the animal.<sup>6</sup> A recent study examined the most common causes of increased iCa concentration in the dog. However, this study was performed by a reference laboratory, and the investigators did not examine the medical records of each patient.<sup>13</sup> Some studies have attempted to correlate serum Ca concentrations to specific diseases.<sup>14,15</sup> There has been no study to the authors' knowledge that has examined whether specific diseases can be correlated with serum iCa concentrations.

The objective of this study was to identify the most common diseases resulting in ionized hypercalcemia and to determine whether a specific iCa range could predict a specific disease entity. We correlated these findings with total Ca and corrected Ca concentrations. We examined the medical records of all dogs in the study to ensure the accuracy of the diagnoses.

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## Materials and Methods

### Criteria for Case Selection

Medical records of the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania (MJR-VHUP) were searched to identify all dogs with a diagnosis of hypercalcemia between January 1998 and December 2003. The resultant medical records were reviewed by one of the authors (JSM). Dogs were included in the study if they had iCa results from at least 2 replicated analyses that were performed upon admission to MJR-VHUP before any treatments were initiated. Ionized Ca concentration was measured on a venous blood gas analyzer<sup>a</sup> using blood collected anaerobically into tubes containing lithium heparin and analyzed immediately after collection.<sup>16</sup> The ion-selective electrodes for Ca in this machine have been validated for use in the dog.<sup>17</sup> Samples were classified as hypercalcemic if results were  $>1.33$  mmol/L (reference range, 1.13–1.33 mmol/L). Records were further reviewed and only those dogs with a definitive diagnosis for the cause of their ionized hypercalcemia were included in the study. Dogs with nonpathologic (nonfasting samples, physiologic growth of young dogs, laboratory error), spurious (lipemia, detergent contamination), or transient (hemoconcentration, severe hypothermia, metabolic acidosis) causes of hypercalcemia were excluded.

### Data Collection

Signalment, pertinent history (including diet history), clinical signs, clinicopathologic findings, endocrine test results, microbial culture findings, cytologic, and histopathologic data, and imaging results were recorded. All testing was performed at the MJR-VHUP clinical pathology and microbiology laboratories except as noted. Intact PTH concentration was determined using a 2-site immunoradiometric assay.<sup>b</sup> vitamin D concentrations were determined for 25-hydroxyvitamin D<sub>3</sub> and 1,25-OH-vitamin D<sub>3</sub> by radioimmunoassay.<sup>b</sup> One of 3 protocols was used for the ACTH stimulation test depending on product availability and clinician preference. ACTH gel<sup>c</sup> was administered at a dosage of 2.2 U/kg IM and serum cortisol concentration<sup>d</sup> was measured before and 2 hours after ACTH administration. In a separate protocol, 0.25 mg of a synthetic ACTH<sup>e</sup> or another ACTH product<sup>f</sup> at a dose of 0.125 mg in dogs with body weight  $<15$  kg or 0.25 mg in dogs with body weight  $>15$  kg was administered IV and serum cortisol concentration measured before and 1 hour after injection.<sup>18,19</sup> Total serum Ca concentration was corrected for albumin using the formula: corrected Ca = serum Ca (mg/dL) – serum albumin (g/dL) + 3.5.<sup>7</sup>

### Diagnostic Criteria

Diagnosis of the various diseases was obtained using the following criteria. All dogs had appropriate clinical signs and physical examination findings for their suspected diseases. Neoplasia and bone marrow disease were diagnosed by cytologic evaluation of needle aspirates or histopathologic evaluation of biopsy specimens. Primary hyperparathyroidism was diagnosed by the presence of increased PTH concentration or PTH concentrations in the upper half of the reference range in the face of an increased iCa. Renal failure was diagnosed if urine specific gravity was 1.005–1.018 and azotemia was present with normal hydration, or azotemia persisted for several days after rehydration. Acute renal failure was diagnosed based on the presence of azotemia with inadequate urine production even after rehydration and absence of urinary obstruction. Vitamin D toxicity was diagnosed with appropriate history of ingestion of vitamin D-containing rodenticide compounds and increased serum 25-hydroxyvitamin D<sub>3</sub> and 1,25-OH-vitamin D<sub>3</sub>.<sup>b</sup> Nonmalignant neoplasia and granulomatous diseases were diagnosed by cytology and histopathology of needle aspirates or biopsy samples of the diseased tissues.

### Statistical Analysis

Statistical analysis was performed using Statistical Analysis System.<sup>20</sup> Continuous dependent variables were analyzed by a one-way analysis of variance (ANOVA) to assess the effect of disease (ie, diagnosis). Differences between continuous dependent variable means were determined by Waller-Duncan *k*-ratio *t*-test.

### Results

One-hundred and fifty-seven dogs were identified that had ionized hypercalcemia. Of those, 109 dogs with a confirmed diagnosis were identified. The mean age of the hypercalcemic dogs was 7.5 years with a range of 6 months to 16 years. Forty-six (42%) of the affected dogs were spayed females, 8 (7%) were intact females, 40 (37%) were neutered males, and 15 (14%) were intact males. A total of 46 different breeds of dogs were represented with the most common being mixed breeds (25%) followed by Golden Retrievers (13%). All other breeds represented  $<4\%$  of the total population. The mean serum iCa concentration was  $1.60 \pm 0.20$  mmol/L, and the median was 1.60 mmol/L. The mean serum total Ca concentration was  $13.57 \pm 2.39$  mg/dL, with a median of 13.45 mg/dL. When serum total Ca concentration was corrected for serum albumin concentration, the mean was  $13.95 \pm 1.95$  mg/dL with a median of 13.50 mg/dL. The interassay coefficient of variation for the iCa measurement was 0.9%.<sup>16</sup> In those dogs in which a diagnosis was achieved, the most common cause of hypercalcemia was neoplasia (58%) (Table 1). Lymphosarcoma was the most common tumor diagnosed and represented 78% of

**Table 1.** Diagnosis of 109 dogs with hypercalcemia.

Diagnosis Group	N	Percentage of Total
Neoplasia	63	58
Lymphosarcoma	49	78 <sup>a</sup>
Carcinoma (CA)	7	11 <sup>a</sup>
Transitional cell CA	1	
Hepatocellular CA	1	
Multiple organ neoplasia	2	
Pulmonary CA	1	
Adrenal CA	1	
Mammary gland epithelial	1	
Anal sac adenocarcinoma	4	6 <sup>a</sup>
Neoplasia—non CA	3	5 <sup>a</sup>
Plasma cell tumor	2	
Osteosarcoma	1	
Renal failure (RF)	18	17
Chronic RF	16	89 <sup>b</sup>
Acute RF	2	11 <sup>b</sup>
Hyperparathyroidism	14	13
Hypoadrenocorticism	6	5
Non malignant neoplasia/granulomatous	5	4
Lipoma	1	
Multiorgan lymph granulomas	1	
Pyogranulomatous disease	1	
Thymoma	1	
Hepatoma	1	
Vitamin D toxicity	3	3

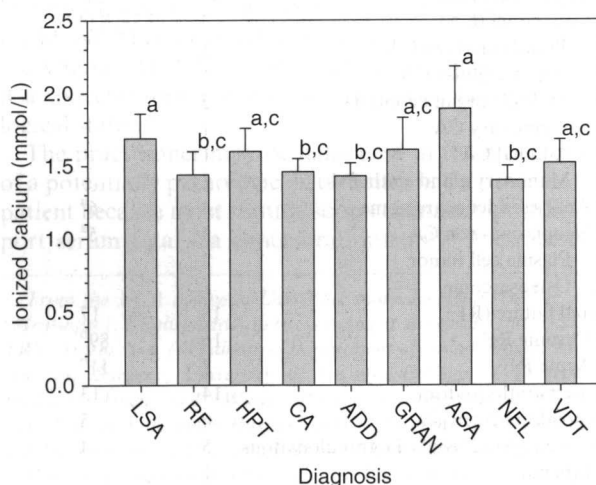
<sup>a</sup>Percentage of neoplasia diagnosis total.

<sup>b</sup>Percentage of renal failure diagnosis total.

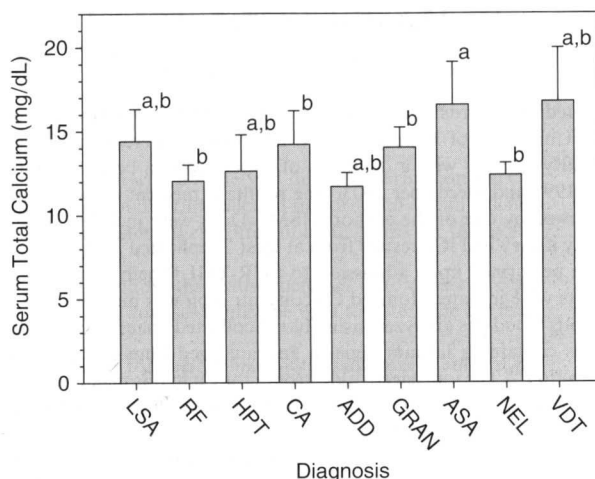
the neoplasia diagnosis group. Two of the dogs with lymphosarcoma also had renal failure. Carcinomas represented the next most common tumors (11%). These tumors included a variety of types: transitional cell carcinoma (1), hepatocellular carcinoma (1), pulmonary carcinoma (1), adrenal carcinoma (1), and mammary gland epithelial cell carcinoma (1). The dog with mammary gland epithelial cell carcinoma also had chronic renal failure. Two dogs had multiple organ neoplasia including thyroid carcinoma and pheochromocytoma (1) and thyroid carcinoma and chondrosarcoma (1). Four dogs (6%) had anal sac adenocarcinoma. Other dogs with neoplasia resulting in hypercalcemia (5%) included 2 dogs with plasma cell tumors and 1 dog with an osteosarcoma.

After neoplasia, then next most common diagnosis was renal failure (17%); these cases included 16 dogs with chronic renal failure and 2 dogs with acute renal failure (Table 1). Dogs with hyperparathyroidism represented 13%, hypoadrenocorticism 5%, and vitamin D toxicity 3% of the population, respectively. Dogs diagnosed with nonmalignant neoplasia or granulomatous disease represented 4% of the population of study animals with increased serum iCa concentrations. This group included 1 dog each with an infected lipoma, multiorgan lymphoid granulomatous disease, pyogranulomatous disease, benign thymoma, and hepatoma.

When serum iCa concentrations were compared among disease groups, dogs with lymphosarcoma and anal sac adenocarcinoma had higher concentrations ( $P < .05$ ) than those with renal failure, carcinoma, hypoadrenocorticism, and noncarcinoma neoplasia (Fig 1). No other significant differences in serum iCa concentrations in the diseased states were found. Only 37% of the variability in serum iCa concentrations could be accounted for by the differences among diseases. When



**Fig. 1.** Mean serum-ionized calcium concentrations for hypercalcemic diseases. Bars with unlike superscripts differ ( $P < .05$ ) LSA, lymphosarcoma; RF, renal failure; HPT, hyperparathyroidism; CA, carcinoma; ADD, hypoadrenocorticism (Addison's disease); GRAN, nonmalignant neoplasia/granulomatous disease; ASA, anal sac adenocarcinoma; NEL, noncarcinoma neoplasia; VDT, vitamin D toxicity.



**Fig. 2.** Mean serum total calcium concentrations for hypercalcemic diseases. Bars with unlike superscripts differ ( $P < .05$ ) LSA, lymphosarcoma; RF, renal failure; HPT, hyperparathyroidism; CA, carcinoma; ADD, hypoadrenocorticism (Addison's disease); GRAN, Nonmalignant neoplasia/granulomatous disease; ASA, anal sac adenocarcinoma; NEL, noncarcinoma neoplasia; VDT, vitamin D toxicity.

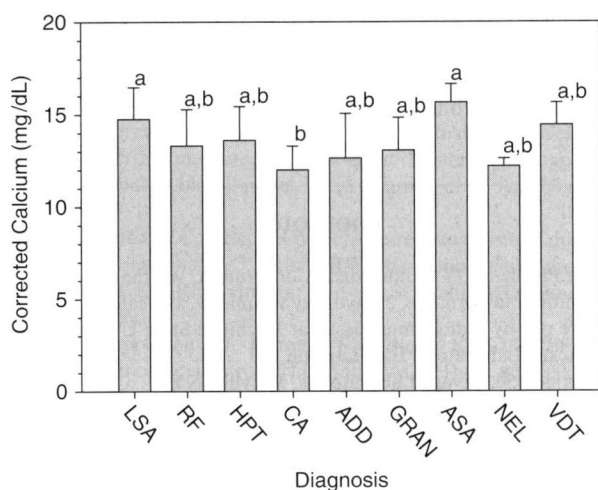
serum total Ca concentrations were compared among disease groups, anal sac adenocarcinoma was found to be associated with higher concentrations ( $P < .05$ ) than in those animals with renal failure, nonmalignant neoplasia or granulomas, carcinoma, and noncarcinomatous neoplasia (Fig 2). All other disease states showed no significant differences in concentrations among one another. Only 22% of the variability in total serum Ca concentrations could be accounted for by the differences among diseases.

Corrected serum Ca concentrations also were compared among disease groups. Animals with lymphosarcoma and anal sac adenocarcinoma had higher ( $P < .05$ ) corrected Ca concentrations than did animals with carcinoma. All hypercalcemic animals with other diseases had concentrations that did not show significant difference (Fig 3). Only 26% of the variability in corrected serum Ca concentrations could be accounted for by the differences among diseases.

## Discussion

In this study, neoplasia was the most common cause of ionized hypercalcemia. This finding is in agreement with several studies that document malignancy as the most common cause of hypercalcemia in dogs.<sup>9,11-13,15</sup> Mechanisms associated with hypercalcemia of malignancy include humoral hypercalcemia of malignancy (HHM), hematologic malignancies growing within the bone marrow, and hypercalcemia caused by metastasis of solid tumors to the skeleton.<sup>21,22</sup> In our study, lymphoma was the most common neoplasm associated with ionized hypercalcemia followed by various sites of carcinoma, anal sac adenocarcinoma, and noncarcinoma neoplasia. The finding of lymphoma as the most common type of neoplasm associated with hypercalcemia is consistent with





**Fig. 3.** Mean corrected calcium concentrations for hypercalcemic diseases. Bars with unlike superscripts differ ( $P < .05$ ). LSA, lymphosarcoma; RF, renal failure; HPT, hyperparathyroidism; CA, carcinoma; ADD, hypoadrenocorticism (Addison's disease); GRAN, nonmalignant neoplasia/granulomatous disease; ASA, anal sac adenocarcinoma; NEL, noncarcinoma neoplasia; VDT, vitamin D toxicity.

previous studies.<sup>9,11,23-25</sup> Approximately 20–40% of dogs with lymphoma are hypercalcemic.<sup>22,26-29</sup> The mechanism of hypercalcemia in dogs with lymphoma is either secondary to tumor production of PTHrP (HHM) or by increased osteoclastic resorption in bones. T-cell lymphoma most often is associated with HHM.<sup>30</sup>

Of the 49 dogs diagnosed with lymphoma and concurrent ionized hypercalcemia in our study, 5 had total Ca concentrations within the normal range. These data are in contrast to a previous study of 16 dogs with lymphoma that showed no strong correlation between iCa and total Ca concentrations in dogs with lymphoma and in normal dogs.<sup>31</sup> In that study, it was concluded that iCa does not provide a diagnostic advantage over the measurement of total Ca in detecting hypercalcemic lymphoma patients. Our study illustrates the importance of iCa measurements to appropriately assess the Ca status in lymphoma patients.

Several studies have shown that dogs with hypercalcemia and lymphoma have T-cell lymphoma.<sup>30,32</sup> Dogs with T-cell lymphoma have been shown to have both shorter remission times and survival times,<sup>24</sup> although there is no prognostic disadvantage in hypercalcemic lymphoma patients.<sup>33,32</sup> Although immunophenotyping was not performed routinely on our patients with lymphoma, the finding of ionized hypercalcemia in our 5 patients with normal total Ca may have indicated underlying T-cell lymphoma, and possibly would have resulted in a worse prognosis for these patients.

Renal failure was the second most common cause of ionized hypercalcemia in our study. Although total serum hypercalcemia in dogs with chronic renal failure is likely the 2nd or 3rd most common cause of hypercalcemia in dogs,<sup>34</sup> iCa concentrations often are normal or subnormal in dogs with chronic renal failure. Less than 10% of all dogs with chronic renal failure have increased

iCa concentrations,<sup>34</sup> and <6% showed ionized hypercalcemia in another report.<sup>35</sup> Increased serum total Ca concentration occurs in up to 14% of dogs with chronic renal failure.<sup>12,36</sup>

In dogs with chronic renal failure, serum total Ca measurement incorrectly assessed iCa status of 36% of dogs, and the use of the Ca adjustment formula for total Ca incorrectly assessed ionized Ca in approximately 53% of dogs with chronic renal failure.<sup>6</sup> Adjusted Ca formulas are suspected to overestimate hypercalcemia due to the lack of considering Ca complexed to other ions such as phosphate, bicarbonate, citrate, and sulfate. Our findings support the importance of measuring iCa in determining the active Ca status in the dogs with chronic renal failure.

Primary hyperparathyroidism was the 3rd most common disease associated with ionized hypercalcemia in our study and was the consequence of parathyroid adenomas or adenomatous hyperplasia resulting in inappropriately increased PTH concentrations. Hypoadrenocorticism was the 4th most common disease causing ionized hypercalcemia. Hypoadrenocorticism previously has been reported as the second most common cause of hypercalcemia in dogs, affecting between 11 and 45% of all cases.<sup>9,11,12,23,37</sup> Recently, ionized hypercalcemia was documented in 18% of dogs with hypoadrenocorticism and found to be predominantly associated with metabolic acidosis.<sup>38</sup>

The mean age for hypercalcemic dogs in our study was 7.5 years, which was expected due to the fact that most diseases associated with hypercalcemia in dogs are found in an older population. Dogs with lymphoma and chronic renal failure are middle aged to older, dogs with anal sac adenocarcinoma have a mean age of 10 years at time of diagnosis, and the mean age of dogs with hyperparathyroidism is 10.5 years.<sup>15,39-41</sup> The exception to these diseases are hypoadrenocorticism and vitamin D toxicity. Most toxicities are associated with younger dogs, and dogs with hypoadrenocorticism are younger to middle aged with a mean age of 4–6 years.<sup>37</sup> The combination of these diseases accounts for the range of ages in our population of dogs with ionized hypercalcemia (6 months to 16 years of age).

Few dogs in our study were intact females (7%) or intact males (14%), which is consistent with the more routine practice of spaying and neutering in our urban study population. In total, 49% of the dogs with hypercalcemia were females and 51% were males. Most diseases associated with hypercalcemia in dogs do not have a sex predilection, aside from hypoadrenocorticism which has been reported to have a female predilection.<sup>37</sup>

Approximately 25% of our study population consisted of mixed breed dogs followed by 13% Golden Retrievers. The high frequency of Golden Retrievers with ionized hypercalcemia was not surprising, because Golden Retrievers are at increased risk for developing lymphoma as well as hyperparathyroidism.<sup>15,42</sup>

In comparing serum iCa concentrations in dogs with different diseases, dogs with lymphoma and anal sac adenocarcinoma in our study had significantly increased concentrations of iCa when compared with those with renal failure, carcinoma, hypoadrenocorticism, and non-

carcinoma neoplasia. However, the concentrations were not significantly higher than those dogs with primary hyperparathyroidism, granulomatous disease or vitamin D toxicity. These data agree in part with a smaller study in which dogs ( $n = 30$ ) with increased serum total Ca concentrations had iCa concentrations measured. The results showed that those with neoplasia (type not reported) and hyperparathyroidism had higher iCa concentrations compared with dogs with chronic renal failure, many of which had normal serum iCa concentrations.<sup>13</sup>

Our results showed less differentiation when serum total Ca concentrations were compared. Significance was only found in dogs with anal sac carcinoma as compared with carcinoma (of other sources), granulomatous disease and noncarcinoma neoplasia. These results contrast with a previous study in which dogs with neoplasia had a higher serum total Ca concentrations than those with primary hyperparathyroidism, vitamin D toxicosis, chronic renal failure, and hypoadrenocorticism.<sup>12</sup> It also disagrees with a previous study in which serum total Ca concentrations were higher in dogs with lymphoproliferative disorders versus those with hypoadrenocorticism<sup>9</sup> and the suggestion that dogs with primary hyperparathyroidism, lymphosarcoma, and anal sac adenocarcinoma have higher serum total Ca concentrations than do dogs with chronic renal failure and vitamin D toxicosis.<sup>14</sup> The differences in our data may be due to our larger case numbers and separation of different types of neoplasia.

When serum Ca concentrations that were corrected for serum albumin were compared, results also were different from those found when serum iCa concentrations were compared. The corrected Ca concentration showed distinction only between dogs with anal sac adenocarcinoma and lymphosarcoma over carcinoma. These data further emphasize the inaccuracies of using the correction formula for Ca.<sup>6</sup>

Limitations of our study include the possibility that some hypercalcemic animals were missed within our database. Files that were mislabeled or not charged for NOVA testing were not included. Another limitation of this study is the relatively high numbers of dogs with neoplasia compared with other diseases associated with ionized hypercalcemia included in the study population. The severity of ionized hypercalcemia may have been higher in dogs with diseases other than neoplasia had the number of dogs with hyperparathyroidism, vitamin D toxicity, hypoadrenocorticism, or granulomatous disease been higher. iCa concentrations were measured at admission to the hospital. Because the referral nature of the caseload, however, some animals received treatments that may have affected their iCa concentrations before admission. The distribution of cases also represents those seen at a referral hospital and may differ from those seen in general practice.

In conclusion, anal sac adenocarcinoma and lymphoma showed significantly higher iCa concentrations when compared with renal failure, carcinoma, hypoadrenocorticism, and noncarcinoma neoplasia. However, there were no other significant differences among groups. Additionally, with the amount of crossover, it is difficult to predict a specific disease based on the severity

of ionized hypercalcemia. Therefore, a complete history, physical examination, and laboratory evaluation are essential for obtaining a definitive diagnosis for diseases associated with ionized hypercalcemia in dogs.

## Footnotes

<sup>a</sup> Stat Profile, NOVA Biomedical Corporation, Waltham, MA

<sup>b</sup> Parathyroid hormone, 25-hydroxy vitamin D, and calcitriol assays were run by radioimmunoassay at Michigan State University Diagnostic Laboratory, East Lansing, MI

<sup>c</sup> Cortigen-50, Savage Laboratories, Melville, NY

<sup>d</sup> Immulite, DPC, Los Angeles, CA

<sup>e</sup> Cortrosyn, Amphstar Pharmaceuticals, Rancho Cucamonga, CA

<sup>f</sup> Synacthen, Alliance Pharmaceuticals Limited, Chippenham, Wiltshire, UK

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